



Aalborg Universitet

AALBORG UNIVERSITY  
DENMARK

## A point mutation in the xylose reductase gene increases citric acid production in *Aspergillus carbonarius*

Weyda, Istvan; Lübeck, Mette; Lübeck, Peter Stephensen

*Publication date:*  
2013

*Document Version*  
Early version, also known as pre-print

[Link to publication from Aalborg University](#)

*Citation for published version (APA):*  
Weyda, I., Lübeck, M., & Lübeck, P. S. (2013). *A point mutation in the xylose reductase gene increases citric acid production in Aspergillus carbonarius*. Poster presented at 35th Symposium on Biotechnology for Fuels and Chemicals, Portland, Oregon, United States.

### General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal -

### Take down policy

If you believe that this document breaches copyright please contact us at [vbn@aub.aau.dk](mailto:vbn@aub.aau.dk) providing details, and we will remove access to the work immediately and investigate your claim.



# A point mutation in the xylose reductase (XR) gene increases citric acid production in *A. carbonarius*

István Weyda, Mette Lübeck and Peter S. Lübeck

Section for Sustainable Biotechnology, Aalborg University Copenhagen, Denmark

## Introduction:

Pentose sugars are among the major components of plant based lignocellulosic biomass. Future biorefineries, which employ plant materials as feedstock, require capable microbial strains for the cost effective conversion of all the sugars of the biomass, including the pentose fraction. Microorganisms metabolize xylose through the pentose catabolic pathway (PCP). In *Aspergillus carbonarius*, the conversion of xylose to xylitol is carried out by a NADPH dependent xylose reductase (XR). Xylitol is further converted by the NAD<sup>+</sup> specific xylitol dehydrogenase. The cofactor impairment between the two enzymes leads to the accumulation of xylitol in xylose fermentation with *A. carbonarius*.

## Materials and methods:

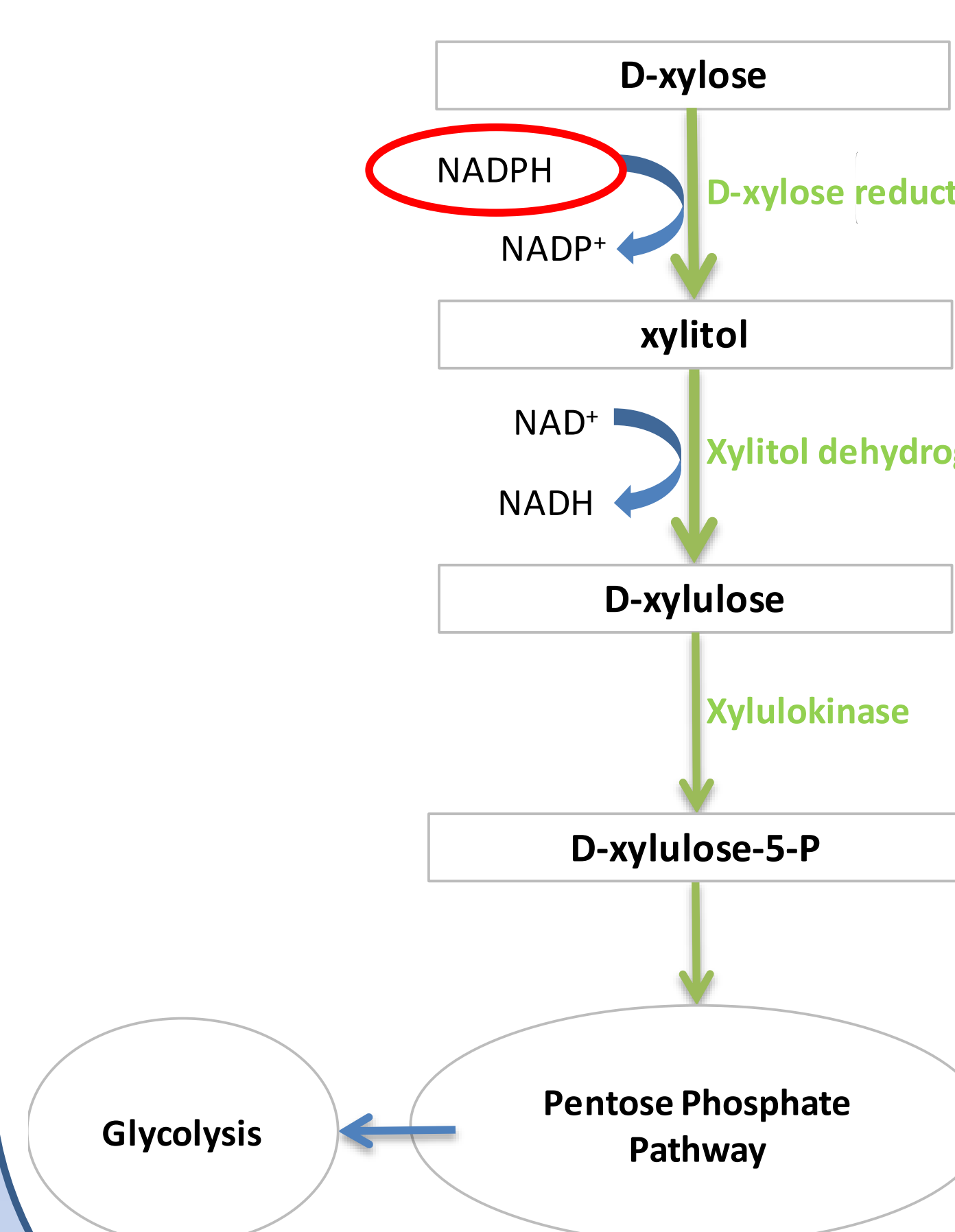
- Cloning and fusion of DNA fragments by USER cloning techniques
- Strain: *Aspergillus carbonarius* ITEM 5010
- Mutation of XR by overlap extension method
- Protoplast transformation using PCR-based bipartite substrates for gene targeting
- Batch flask fermentation on defined D-xylose medium in rotary shaker for 7 days.

## Purpose of the project:

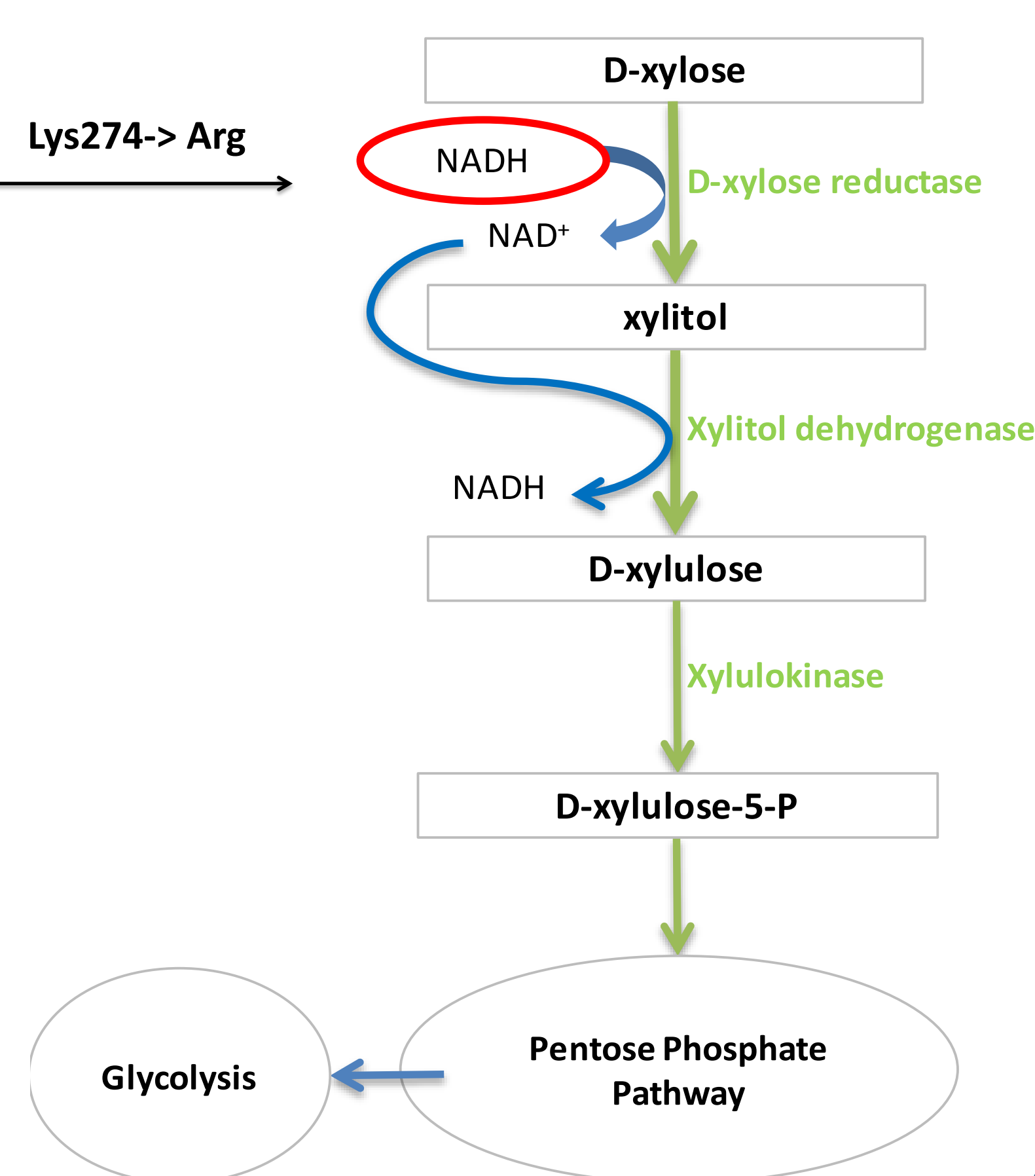
The aim is to assure the flux of D-xylose through the PCP by preventing the accumulation of pathway intermediates.

In order to overcome xylitol accumulation, the XR gene of *A. carbonarius* was identified and the cofactor preference was altered from NADPH to NADH, by introducing a point mutation (Lys274 to Arg) into the enzyme by site-directed mutagenesis.

### Wild-type strain



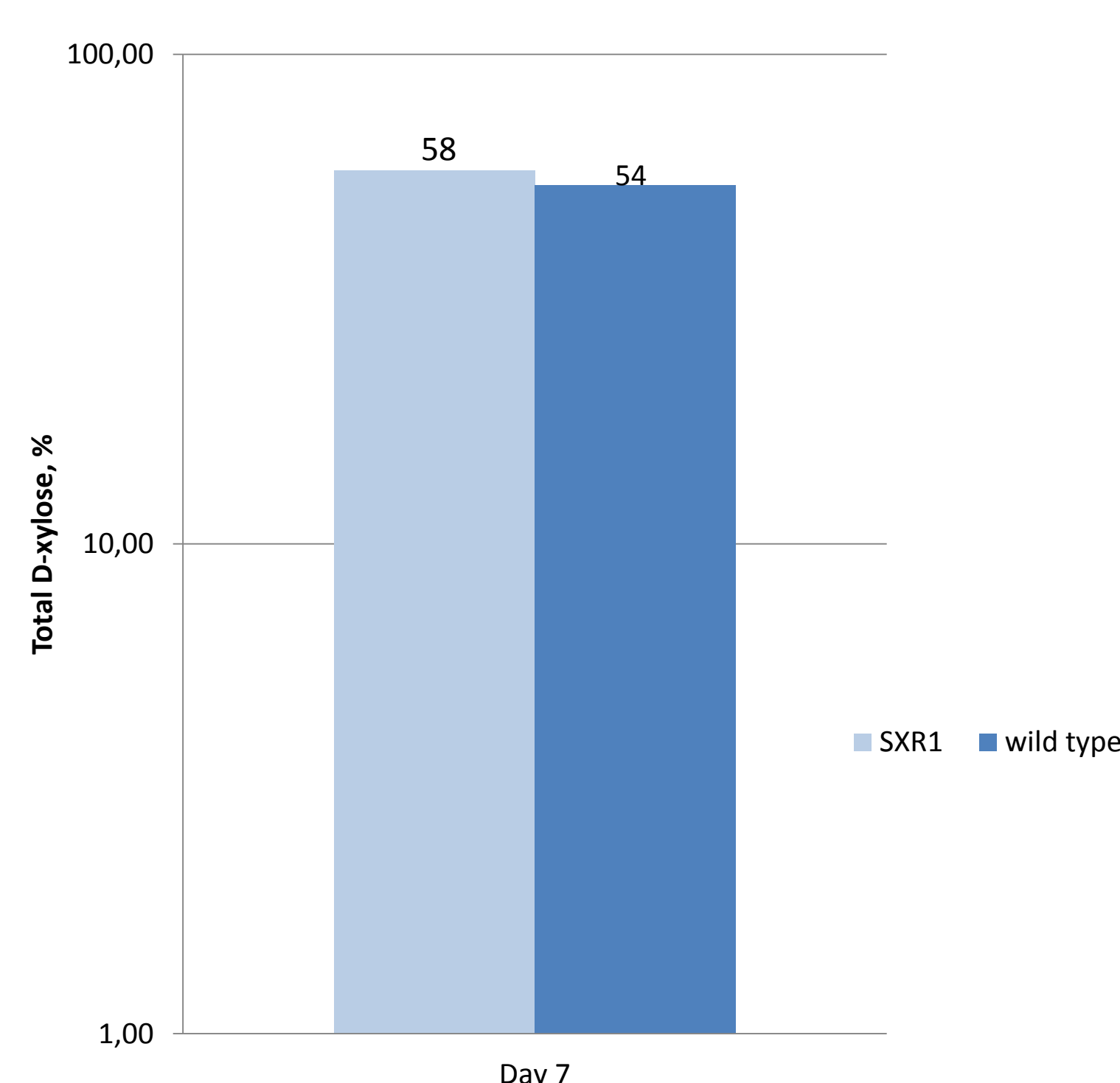
### Mutant strain



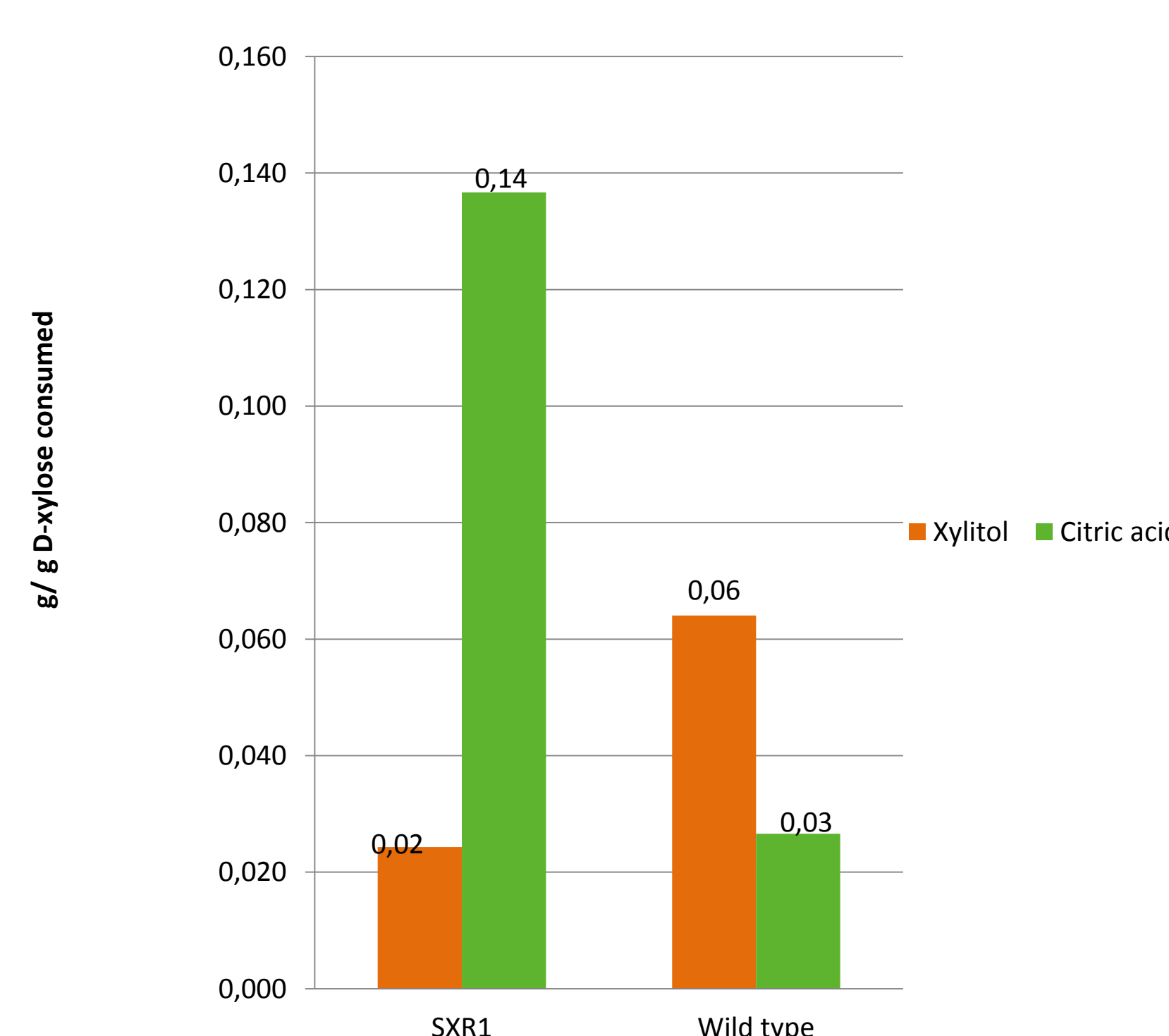
## Results and Conclusion:

The results of the fermentation conducted on defined D-xylose medium with the mutant (SXR1) and wild type strain showed:

- The point mutation in the XR did not affect the rate of D-xylose consumption (Figure 1.)
- Citric acid yield increased 5 fold, while xylitol accumulation was reduced 2.5 fold in SXR1 (Figure 2.)



**Figure 1.** D-xylose consumption of mutant (SXR1) and wild type strain at day 7 of the fermentation, expressed in percentage of initial D-xylose



**Figure 2.** Yield of citric acid and xylitol by mutant (SXR1) and wild type strain at day 7 of the fermentation

## References:

<sup>1</sup>Witteveen CFB, Busink R, Vandevondervoort P, Dijkema C, Swart K, Visser J (1989) L-Arabinose and D-Xylose Catabolism in *Aspergillus niger*. J Gen Microbiol 135:2163-2171

<sup>2</sup>Leitgeb S, Petschacher B, Wilson DK, Nidetzky B (2005) Fine tuning of coenzyme specificity in family 2 aldo-keto reductases revealed by crystal structures of the Lys-274-->Arg mutant of *Candida tenuis* xylose reductase (AKR2B5) bound to NAD<sup>+</sup> and NADP<sup>+</sup>. FEBS letters 579:763-767.